*Biochimica et Biophysica Acta*, 551 (1979) 197—206


BBA 78282

# KINETICS OF SULFATE UPTAKE BY YEAST

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(Received June 20th, 1978)

*Key words: Sulfate uptake; Ion transport; Surface potential; Cotransport; (Yeast)*

## Summary

Uptake of sulfate by yeast requires the presence of a metabolic substrate and is dependent on the time during which the cells have been metabolizing in the absence of sulfate. At low concentrations of sulfate, uptake can be described by simple saturation kinetics. Uptake of sulfate is accompanied by a net proton influx of 3 $H^+$ and an efflux of 1 $K^+$ for each sulfate ion taken up. Divalent cations stimulate sulfate uptake at low concentrations of sulfate; the maximal rate of uptake is not significantly affected but $K_m$ is lowered. Stimulation by divalent cations shows an optimum at a cation concentration of about 4 mM. Monovalent cations are less effective, trivalent cations are more effective in stimulating sulfate uptake. The results are qualitatively in accordance with the notion, that the effect of cations is due to an effect via the surface potential.

## Introduction

The kinetics of sulfate uptake have been relatively well studied in *Penicillium* [1—3] and preliminary studies have been performed on *Saccharomyces* [4,5]. Cuppoletti and Segel [3] found a dependence of sulfate uptake on the pH of the suspending medium and on the $Ca^{2+}$ concentration. Based on kinetical experiments a model for sulfate uptake in *Penicillium* was developed, that described the uptake process at a cotransport of one sulfate ion with one $H^+$ and one $Ca^{2+}$. Since $Ca^{2+}$ was not accumulated in a 1 : 1 stoicheometry with

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sulfate, it was assumed that most of the translocated $Ca^{2+}$ would return with the unloaded carrier to the external side of the membrane.

Theuvenet and Borst-Pauwels [6,7] suggested a different mechanism by which multivalent cations might stimulate uptake of anions. A reduction of the surface potential by the addition of multivalent cations will favour the accumulation of anions at the membrane-solution interface and thus enhance the rate of anion uptake. If, however, the anion is taken up via a cotransport mechanism with cations, the effect of the surface potential on the transport kinetics of such a cotransport system will be complex, since the interfacial concentrations of anions and cations are affected in an opposed way [8]. We will show, that the effects of cations on sulfate uptake can at least partly be attributed to an effect via the surface potential.

## Materials and Methods

Yeast cells, *Saccharomyces cerevisiae* strain Delft II, with a low phosphate content, were starved in distilled water under aeration for 20 h. The aeration period was kept constant, since prolonged aeration resulted in a decrease of the rate of sulfate uptake. After starvation, the cells (2%, w/v) were incubated for 2 h in 45 mM Tris/succinate buffer of the desired pH, in the presence of 3% (w/v) glucose at 25°C. Nitrogen was bubbled through the suspension continuously. The uptake of sulfate (added to the medium as Tris/sulfate) was studied using $^{35}SO_4$ as a tracer, with the technique described earlier for phosphate uptake [9]. The filters were not dried, and the radioactivity was determined by means of liquid scintillation analysis. Initial uptake rates were determined from the slopes of the tangents to the uptake curves at zero time. Uptake of $Ca^{2+}$ was determined as described elsewhere [10].

Efflux of $K^+$ was measured with a $K^+$-selective electrode (Philips IS 560) in a buffered suspension. Proton fluxes were measured in unbuffered suspension by means of a pH stat, with triethylamine and HCl as titrants. These experiments were carried out with a 5% (w/v) yeast suspension provided with 7.5% (w/v) glucose, after a preincubation of 2 h at pH 5.3.

Cells with a lower cell pH were obtained by addition of 4 mM butyric acid, adjusted to pH 4.5 with Tris, to the yeast suspension at 6 min prior to uptake. The cell pH was determined after freezing and boiling the cells [11].

Complexation of sulfate by $Ca^{2+}$ and $Cr^{3+}$ was studied with a $Ca^{2+}$-selective electrode (Philips IS 560); the electrode was also sensitive to $Cr^{3+}$. Solutions of $CaCl_2$ and $CrCl_3$ were titrated with Tris/sulfate or $Na_2SO_4$ as described by Kobos and Rechnitz [12].

## Results

Uptake of sulfate requires the presence of a metabolic substrate and is markedly dependent on the time during which the cells have been metabolizing in the absence of sulfate. The rate of sulfate uptake is maximal after about 2 h. At low concentrations of sulfate, up to about 0.5 mM, uptake can be described by simple Michaelis-Menten kinetics (see also Fig. 2). We have only studied the kinetical properties of this high affinity uptake mechanism. Our

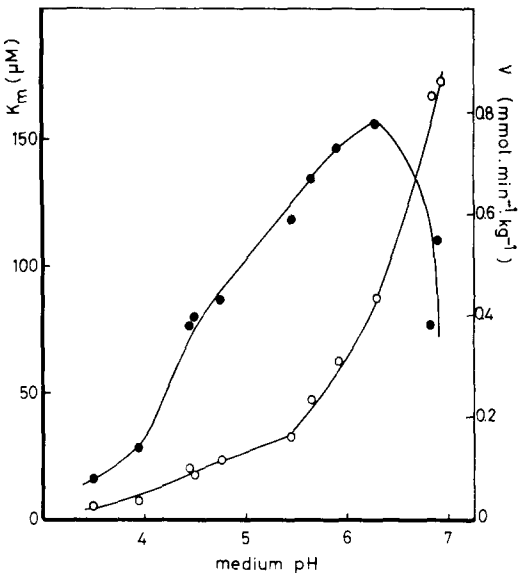



Fig. 1. Effect of the pH of the suspending medium on the kinetical constants of sulfate uptake; ○, $K_m$; ●, $V$.

experimental data do not, however, exclude the possibility that there is also a low affinity sulfate uptake mechanism operative at high concentrations of sulfate, as found by Breton and Surdin-Kerjan [5]. The apparent affinity constant $K_m$ and the maximal rate of uptake $V$ of the high affinity system depend on the pH of the suspending medium as shown in Fig. 1. These results resemble strongly those obtained with phosphate uptake by yeast [13] and show direct or indirect interaction of protons with the sulfate uptake mechanism. Phosphate uptake by yeast is accompanied by net proton influx and extrusion of $K^+$ [14].

Experiments were carried out to determine whether this was also true for sulfate uptake. Tris/sulfate was added to the yeast suspension (pH 5.3) and proton and $K^+$ fluxes were measured. Corrections for the effect of $Tris^+$ on these fluxes were made by adding an equivalent amount of Tris-HCl in a control experiment (Table I). We found that addition of sulfate to the yeast suspension caused an immediate efflux of $K^+$; for each sulfate ion taken up,

TABLE I

$H^+$ AND $K^+$ FLUXES ACCOMPANYING SULFATE UPTAKE BY YEAST

Fluxes are given in mmol · $min^{-1}$ · $kg^{-1}$. Sulfuric acid (0.25 mM in the proton flux experiments, 0.5 mM in the $K^+$ flux experiments), adjusted to pH 5.3 with Tris, was added to the yeast suspension. In the control experiment, the same amount of Tris, adjusted to pH 5.3 with HCl was added to the suspension.

| | Efflux ($SO_4^{2-}$ added) | Efflux (control) | Net flux | $^{v}SO_4^{2-}$ | Ratio |
|---|---|---|---|---|---|
| $H^+$ | 6.6 | 8.4 | 1.8 (in) | 0.53 | 3.4 |
| $K^+$ | 0.60 | 0 | 0.60 (out) | 0.58 | 1.04 |

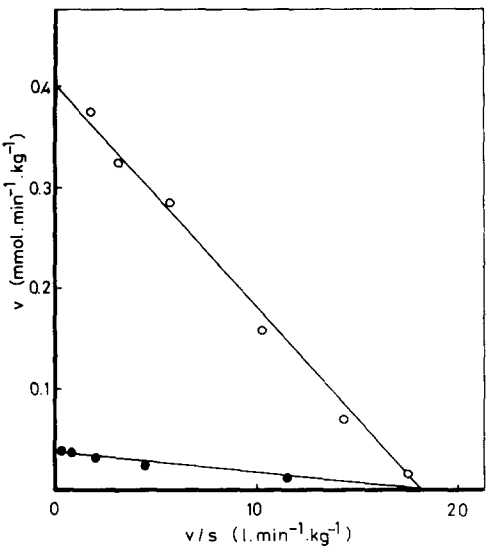



Fig. 2. Effect of the cell pH on the kinetics of sulfate uptake, at a medium pH of 4.5; ○, control, 4 mM Tris-HCl added at 6 min prior to uptake, cell pH 6.73; ●, 4 mM Tris/butyrate added at 6 min prior to uptake, cell pH 6.30.

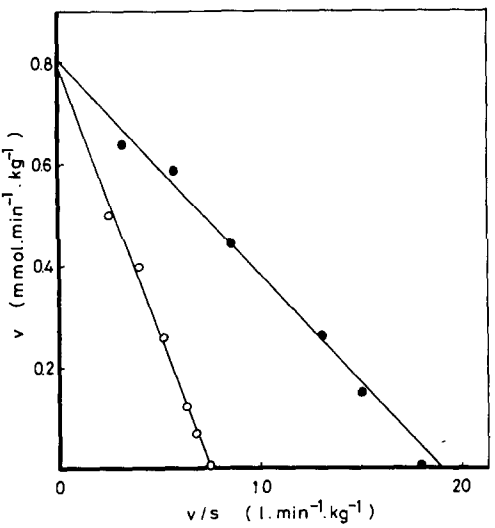
1.04 ± 0.15 $K^+$ were extruded (mean and standard deviation of 5 experiments). The proton fluxes accompanying sulfate uptake were determined in an unbuffered suspension kept at pH 5.3 in a pH stat. From the difference between the $H^+$ efflux in the presence and in the absence of sulfate, a net proton influx of 1.8 mequiv. $H^+$/kg per min caused by sulfate could be calculated. From four paired experiments a ratio of 3.4 ± 0.5 (mean and standard deviation) could be determined. Probably sulfate is cotransported with 3 protons; electroneutrality is maintained by efflux of one $K^+$.

Both $K_m$ and $V$ of sulfate uptake depend on the intracellular pH as shown in Fig. 2 (data represented according to Hofstee [15]). This may point to interaction of intracellular $H^+$ or $OH^-$ with the uptake mechanism. The results with sulfate uptake are similar to those obtained with phosphate uptake [13].

At pH 4.5, sulfate uptake is inhibited by phosphate; at a concentration of 200 $\mu$M phosphate, the maximal rate of uptake is reduced to about 50% of the control value, but $K_m$ is not affected; it is hence unlikely that sulfate and phosphate compete for the same transport site. The dependence of the inhibition on the concentration of phosphate was investigated at low sulfate concentration. The inhibition by phosphate could be described by saturation kinetics; the concentration of phosphate at which half-maximal inhibition of sulfate uptake was found, was about equal to the $K_m$ of phosphate for the phosphate uptake mechanism (11 $\mu$M at pH 4.5).

Sulfate uptake is also inhibited by 2,4-dinitrophenol. At pH 4.5, addition of 0.1 mM dinitrophenol 6 min prior to addition of radioactive sulfate to the yeast suspension, causes a reduction of both $V$ and $K_m$ of sulfate uptake. $V$ is decreased to 0.04 mmol/min per kg (compared to 0.40 mmol/min per kg for the control), and $K_m$ is decreased to 3.5 $\mu$M (compared to 20 $\mu$M for the control).

The effect of $Mg^{2+}$ on sulfate uptake is shown in Fig. 3; it appears that the



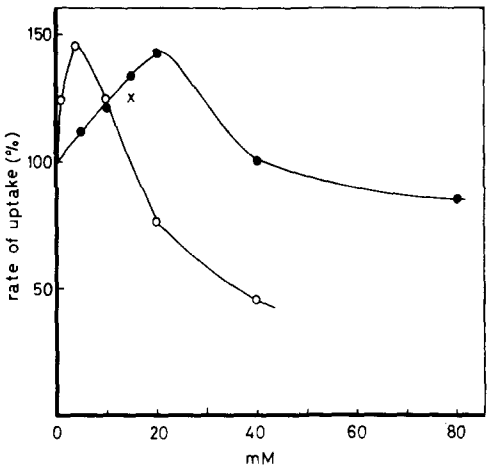




Fig. 3. Effect of $Mg^{2+}$ on the kinetics of sulfate uptake at pH 6.25; ○, control; ●, 4 mM $MgCl_2$ added.

Fig. 4. Effect of $Mg^{2+}$ and $K^+$ on the rate of sulfate uptake at pH 4.5; the sulfate concentration was 1 μM. ○, $Mg^{2+}$; ●, $K^+$. In addition, the stimulation by 15 mM $Li^+$ is given in the figure (X).

stimulating effect of $Mg^{2+}$ is mainly due to a decrease of $K_m$ and that $V$ is hardly affected. If the stimulation by $Mg^{2+}$ is plotted as a function of the $Mg^{2+}$ concentration, an optimum is found, situated at about 4 mM $Mg^{2+}$ (Fig. 4). The stimulating effect is not specific for $Mg^{2+}$, similar results are obtained with other divalent cations, and there is relatively little difference between the effect of various ions (Table II).

Also monovalent cations, of which $K^+$ and $Li^+$ were tested, enhance sulfate uptake, though less effectively than divalent cations. For $K^+$ it was found, that the optimal concentration was at about 20 mM (Fig. 4). On the other hand, trivalent cations, of which we tested $Al^{3+}$ and $Cr^{3+}$, were more effective; the optimum occurred at about 0.4 mM $Cr^{3+}$ (Fig. 5).

Fig. 5 shows, that if sulfate uptake is already maximally stimulated by $Mg^{2+}$, addition of $Cr^{3+}$ does not further enhance the rate of sulfate uptake, but on the contrary has an inhibiting effect. This suggests that the effect of $Mg^{2+}$ and $Cr^{3+}$

TABLE II

EFFECT OF DIVALENT CATIONS ON SULFATE UPTAKE AT pH 4.5

The concentration of sulfate during the experiment was 1 μM. Divalent cations were added to a concentration of 4 mM. Data are mean ± S.D., $n$ = 3.

| | Rate of uptake (%) |
|---|---|
| Control | 100 |
| $Mg^{2+}$ | 157 ± 13 |
| $Ca^{2+}$ | 156 ± 7 |
| $Sr^{2+}$ | 167 ± 4 |
| $Zn^{2+}$ | 160 ± 13 |
| $Mn^{2+}$ | 141 ± 15 |

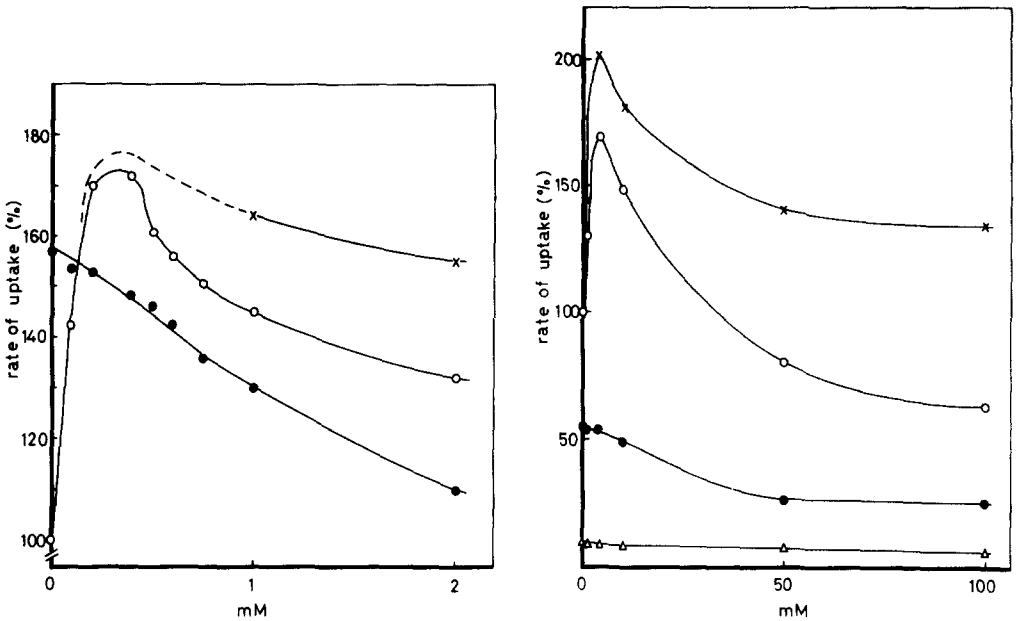



Fig. 5. Effect of $Cr^{3+}$ on the rate of sulfate uptake at pH 4.5 (sulfate concentration 1 $\mu$M); ○, without added $Mg^{2+}$; ●, 4 mM $Mg^{2+}$ added; X, data without added $Mg^{2+}$ corrected for complexation of sulfate by $Cr^{3+}$; below 1 mM $Cr^{3+}$ no accurate data could be obtained.

Fig. 6. Effect of $Ca^{2+}$ on the rate of sulfate uptake; ○, pH 4.5; ●, pH 3.5; △, pH 7.4; X, pH 4.5 corrected for complexation of sulfate by $Ca^{2+}$. The sulfate concentration in the experiments was 1 $\mu$M.

is based on the same mechanism and the result could very well be explained by assuming that once the optimal value of the surface potential is reached, a further reduction, be it by addition of $Mg^{2+}$ or by that of $Cr^{3+}$, will inhibit sulfate uptake. Similarly, in the presence of optimal concentrations of $Mg^{2+}$ or $Cr^{3+}$, addition of 15 mM $K^+$ inhibits sulfate uptake with about 15%, whereas in the absence of $Mg^{2+}$ or $Cr^{3+}$ it stimulates sulfate uptake by 30% (Fig. 4).

Divalent cations only stimulate sulfate uptake at intermediate pH values. At very low (<pH 4) or high (>pH 7) values of pH no stimulation occurs (Fig. 6).

To determine whether the decrease in stimulation of sulfate uptake by divalent or trivalent cations at high concentrations of these ions might be due to complexation of sulfate, the complexation constants of $Ca^{2+}$ and $Cr^{3+}$ with sulfate were measured by titration of solutions of $CaCl_2$ or $CrCl_3$ with sulfate; measurements were carried out in 45 mM Tris/succinate buffer at pH 4.5. In Figs. 5 and 6 the resulting corrections for complexation of sulfate by $Ca^{2+}$ and $Cr^{3+}$ at this pH are shown. The decrease of stimulation of sulfate uptake at high concentrations of $Ca^{2+}$ and $Cr^{3+}$ appears to be only partly due to complexation of sulfate.

Uptake of $Ca^{2+}$ (at 1 $\mu$M $Ca^{2+}$) was not stimulated by sulfate, hence no support was obtained for the notion that sulfate might be cotransported with divalent cations.

## Discussion

In several respects, the kinetics of sulfate uptake by yeast resemble those of phosphate uptake. Both ions appear to be cotransported with protons (or exchanged for cellular $OH^-$). The ratio between anion uptake and proton uptake allows translocation via a positively charged complex and the charge balance is maintained by extrusion of $K^+$, the most abundant cellular cation. In this way, anion uptake may be coupled to the uptake of protons along their electrochemical gradient. The dependency of sulfate uptake on the sulfur starvation period parallels the results obtained with phosphate uptake by yeast [16]. Also the effects of dinitrophenol [17] and divalent cations [7] are qualitatively similar. On the other hand, it is unlikely that the sulfate transport system is identical with the phosphate transport system: phosphate ions do not inhibit sulfate uptake in a competitive way, and sulfate ions do not inhibit phosphate uptake [18].

Our findings strongly suggest that phosphate and sulfate are taken up via similar transport systems. This is also reflected in the pH dependence of phosphate and sulfate uptake. In the case of phosphate, it has been shown that the dependence of the maximal rate of uptake on the pH of the suspending medium is, in fact, only apparent; $V$ depends only on the cell pH [13]. The medium pH at which a maximum for $V$ of sulfate uptake is found, corresponds with a cell pH of 6.95; this is close to the optimal cell pH for $V$ of phosphate uptake, about 6.8. If, indeed, $V$ of sulfate uptake is independent of the extracellular pH, this would point to a transport mechanism in which protons bind to the carrier before the sulfate ion [8] (see Appendix). Such an order of binding has also been suggested for sulfate uptake in *Penicillium* [3]. We will show, that the effects of the surface potential on sulfate uptake are in accordance with such a mechanism.

In a theoretical study of the kinetics of carrier-mediated ion transport, Borst-Pauwels [19] showed, that if the carrier can move freely across the cell membrane, $K_m$ and $V$ are not independent kinetical parameters, but they are interrelated and dependent on the intracellular concentration of ions that have affinity for the carrier. The results indicate that sulfate uptake by yeast, in analogy with phosphate uptake [13] is a carrier-mediated process, and that intracellular $H^+$ or $OH^-$ have affinity to the sulfate carrier.

An inhibition by phosphate, similar to that of sulfate uptake, has also been found with uptake of monovalent and divalent cations by yeast [9,10]. We have suggested, that this inhibition is due to a transient depolarization of the membrane by phosphate. Inhibition of sulfate uptake would then also be expected, if sulfate is translocated as a positively charged complex.

The finding that inhibition of sulfate uptake by 2,4-dinitrophenol is strongest at high concentrations of sulfate is similar to results obtained with phosphate uptake [17]. The inhibitory effect of dinitrophenol is probably not only due to depolarization, but also to a decrease of the cell pH. Earlier observations [20] showed that under anaerobic conditions, 2,4-dinitrophenol caused only a minor decrease in cell ATP level.

The effect of fatty acids and especially of butyric acid on phosphate uptake has been studied extensively [13,20,21] and it was concluded [21] that the

effect of butyric acid and other fatty acids is only an indirect effect via the cell pH.

In a theoretical study [8] we have shown, that if an anion is taken up via a cotransport mechanism with cations, the rate of uptake of the anion may show an optimum at a certain value of the surface potential. Consequently, if the surface potential is changed by addition of a cation, the rate of anion uptake may show an optimum if plotted as function of the concentration of this cation. Qualitatively this may be understood by assuming that at a low concentration of this cation, the effect of the increase of the interfacial anion concentration would be predominating, but that at high concentrations of the cation used to decrease the surface potential, the decrease of the interfacial concentration of the positively charged cosubstrate, in this case $H^+$, would become more important.

Qualitatively, the effect of cations on sulfate uptake can be described by an effect via the surface potential. The finding that trivalent cations are one order of magnitude more effective than divalent cations, and these again more effective than monovalent cations is in accordance with this notion. In the model proposed for sulfate uptake, only $K_m$ would be affected by changes in the surface potential, not $V$ (see Appendix, Eqn. A3). This may explain why only the $K_m$ of sulfate uptake is markedly affected by $Mg^{2+}$. Under certain conditions, namely when $K_{j1}K_{j2}K_{j3} >> s_j^3 y^3$, $K_m$ will show a minimum if plotted as a function of $y$ or of the surface potential (see Appendix, Eqn A4). These conditions will be fulfilled if at least one of the cation binding sites has a relatively low affinity for protons. In that case a minimum in $K_m$ may be expected as a function of the concentration of an ion by which the surface potential is affected, and this will give rise to plots as seen in Figs. 4—6.

Alternative explanations of the stimulation of sulfate uptake by cations have been considered. Divalent and trivalent cations cause hyperpolarization and this could contribute to the observed stimulation of sulfate uptake. On the other hand, $K^+$, which causes depolarization, also stimulates sulfate uptake. $K^+$ might stimulate sulfate uptake indirectly by increasing the cell pH, but then it cannot be explained why $K^+$ inhibits sulfate uptake in the presence of optimal concentrations of $Mg^{2+}$ or $Cr^{3+}$, which do not affect the cell pH appreciably. Neither can the effect of $Li^+$ be explained as an effect via the cell pH. The decrease of stimulation at higher cation concentration is not exclusively due to complexation of sulfate.

Although effects via membrane potential, cell pH and complexation may contribute to the observed effects of cations on sulfate uptake, and this may be reflected in the shape of the curves in Figs. 4—6, the most probable explanation appears to be that the effect of cations on sulfate uptake are mainly due to changes in the surface potential.

The absence of stimulation of sulfate uptake at very low pH may be due to the fact that at this pH the surface potential is so small, that no further decrease can be obtained by the addition of divalent cations. We have shown theoretically [8] that stimulation of anion uptake by a decrease of the surface potential may be absent or much smaller at very low concentrations of the cosubstrate, in this case at high pH.

Various characteristics of sulfate uptake by yeast, described here, have also

been found with other organisms. The dependency of sulfate uptake on the sulfur starvation period, possibly reflecting the synthesis of a sulfate-binding or a sulfate-transporting protein, has been found in various fungi and algae [1,22–25]. A pH optimum for sulfate uptake in the range of 6.0–6.3 has been found in fungal and plant cells [1,26]. Inhibition of sulfate uptake by dinitrophenol has been observed in fungi, algae and higher plants [1,26,27]. Stimulation of sulfate uptake by cations also occurs in other fungi and plant cells. The optimal concentration of $Ca^{2+}$ has been found to be 1–3 mM in barley roots [28]. It has also been shown, that monovalent cations are less effective in stimulating sulfate uptake, but that trivalent cations are more effective [2,3]. Only the inhibitory effect of phosphate found in this study, appears to be in contradiction with the literature [23,27]. If our explanation of the inhibition is correct, in that it is due to a transient depolarization caused by phosphate uptake via the $H^+$-phosphate cotransport mechanism [9], inhibition would then only be found in phosphate-deficient cells (in which this mechanism is active) and only in short-term experiments.

It may well be possible, that the mechanism for sulfate uptake as proposed here, is not limited to yeast, but occurs more wide-spread in nature.

## Appendix

The general rate equation for anion uptake via a transport system by which the anion ($s_i$) is cotransported with three cations ($s_j$) in the case that the cations bind to the translocator before the anion, is given by:

$$v_i = \frac{V \cdot s_i}{K_i\left(\frac{K_{j1}K_{j2}K_{j3} + (K_{j1}K_{j2} + K_{j1}K_{j3} + K_{j2}K_{j3})s_j + (K_{j1} + K_{j2} + K_{j3})s_j^2 + s_j^3}{s_j^3}\right) + s_i}$$

$$= \frac{V \cdot s_i}{K_{m,i} + s_i} \qquad \text{(A1)}$$

where $K_i$, $K_{j1}$, $K_{j2}$ and $K_{j3}$ are, in the case of carrier-mediated transport, complex constants, related to the affinity of the anion or cations, respectively, to the transport mechanism [8].

For ion transport across charged membranes, the concentrations of the ions in the aqueous bulk phase ($s$) should be substituted by their concentrations at the membrane-solution interface ($s_0$) which is related to $s$ by the Boltzmann distribution law:

$$s_0 = s \exp(-zq\psi_0/kT) = sy^z \qquad \text{(A2)}$$

where $z$ is the valency of the ion, $q$ the absolute value of the charge of the electron, $\psi_0$ the surface potential, $k$ the Boltzmann constant and $T$ the absolute temperature; $y$ is related to the surface potential and defined by Eqn. A2; for negatively charged membranes $y > 1$.

For a divalent anion and a monovalent cation, Eqn. A1 is transformed into:

$$v_i = \frac{V \cdot s_i}{K_i\left(\frac{K_{j1}K_{j2}K_{j3} + (K_{j1}K_{j2} + K_{j1}K_{j3} + K_{j2}K_{j3})s_j y + (K_{j1} + K_{j2} + K_{j3})s_j^2 y^2 + s_j^3 y^3}{s_j^3 y}\right) + s_i} \qquad \text{(A3)}$$

$V$ is not dependent on $s_j$ (the proton concentration), or $y$, but $K_{m,i}$ is dependent on the surface potential and $s_j$. From Eqn. A4:

$$\frac{dK_{m,i}}{dy} = \frac{-K_i}{s_j^3 y^2}(K_{j1}K_{j2}K_{j3} - (K_{j1} + K_{j2} + K_{j3})s_j^2 y^2 - 2s_j^3 y^3) \quad \text{(A4)}$$

it can be seen that under certain conditions, namely when $K_{j1}K_{j2}K_{j3} >> s_j^3 y^3$, $K_{m,i}$ will have a minimum.

## Acknowledgements

The technical assistance of Mr. C. Weyers and Mr. J. Dobbelmann is gratefully acknowledged. The study was financially supported by the Netherlands Organisation for the Advancement of Pure Research (Z.W.O.) under auspices of the Netherlands Foundation for Biophysics. The yeast was kindly supplied by Gist-Brocades at Delft.

## References

1 Tweedie, J.H. and Segel, I.H. (1970) Biochim. Biophys. Acta 196, 95—106
2 Bradfield, G., Somerfield, P., Meyn, T., Holby, M., Babcock, D., Bradley, D. and Segel, I.H. (1970) Plant Physiol. 46, 720—727
3 Cuppoletti, J. and Segel, I.H. (1975) Biochemistry 14, 4712—4718
4 McCready, R.G.L. and Din, G.A. (1974) FEBS Lett. 38, 361—363
5 Breton, A. and Surdin-Kerjan, Y. (1977) J. Bacteriol. 132, 224—232
6 Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1976) J. Theor. Biol. 57, 313—329
7 Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1976) Bioelectrochem. Bioenerg. 3, 230—240
8 Roomans, G.M. and Borst-Pauwels, G.W.F.H. (1978) J. Theor. Biol. 73, 453—468
9 Roomans, G.M. and Borst-Pauwels, G.W.F.H. (1977) Biochim. Biophys. Acta 470, 84—91
10 Roomans, G.M., Theuvenet, A.P.R., Van den Berg, Th.P.R. and Borst-Pauwels, G.W.F.H. (1979) Biochim. Biophys. Acta 551, 187—196
11 Borst-Pauwels, G.W.F.H. and Dobbelman, J. (1972) Acta Bot. Neerl. 21, 149—154
12 Kobos, R.K. and Rechnitz, G.A. (1976) Arch. Biochem. Biophys. 175, 11—20
13 Borst-Pauwels, G.W.F.H. and Peters, P.H.J. (1977) Biochim. Biophys. Acta 466, 488—495
14 Cockburn, M., Earnshaw, P. and Eddy, A.A. (1975) Biochem. J. 146, 705—712
15 Hofstee, B.H.J. (1952) Science 116, 329—331
16 Roomans, G.M., Blasco, F. and Borst-Pauwels, G.W.F.H. (1977) Biochim. Biophys. Acta 467, 65—71
17 Borst-Pauwels, G.W.F.H. and Huygen, P.L.M. (1972) Biochim. Biophys. Acta 288, 166—171
18 Borst-Pauwels, G.W.F.H. (1964) Biochim. Biophys. Acta 93, 659—661
19 Borst-Pauwels, G.W.F.H. (1974) J. Theor. Biol. 48, 183—195
20 Borst-Pauwels, G.W.F.H. and Jager, S. (1969) Biochim. Biophys. Acta 172, 399—406
21 Borst-Pauwels, G.W.F.H. and Dobbelmann, J. (1972) Biochim. Biophys. Acta 290, 348—354
22 Vallée, M. and Jeanjean, R. (1968) Biochim. Biophys. Acta 150, 599—606
23 Deane, E.M. and O'Brien, R.W. (1975) Arch. Microbiol. 105, 295—301
24 Passera, C. and Ferrari, G. (1975) Physiol. Plant. 35, 318—321
25 Jeanjean, R. and Broda, E. (1977) Arch. Microbiol. 114, 19—23
26 Persson, L. (1969) Physiol. Plant. 22, 959—976
27 Wedding, R.T. and Black, M.K. (1960) Plant Physiol. 35, 72—80
28 Legget, J.E. and Epstein, A. (1956) Plant Physiol. 31, 222—226